APPENDIX A
MARKED UP VERSION TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at page 29, line 17, has been replaced with the following rewritten paragraph.

-- The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online at the GCG website ~~at http://www.gcg.com~~), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online at the GCG website ~~at http://www.gcg.com~~), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.--

The bridging paragraph beginning at page 29, line 31, has been replaced with the following rewritten paragraph.

--The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to AS3 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to AS3 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and

Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used, which are available online at the National Center for Biotechnology Information. ~~See http://www.ncbi.nlm.nih.gov.~~--

The following two paragraphs beginning at page 73, line 20, have been replaced with the following rewritten paragraphs:

--Briefly, androgen-specific, low-abundance regulatory mRNA sequences expressed during the proliferative shutoff, were selected using the Wang-Brown approach (Wang *et al.*, (1991) *Proc. Natl. Acad. Sci. USA* 88: 11505-11509). Short fragments of cDNAs were amplified first: then three cycles of subtractions and amplifications between the control and proliferation arrested cDNAs resulted in sequence pools that were differentially expressed (Geck *et al.*, (1997) *J. Steroid Biochem. Mol. Biol.* 63: 211-218). LNCaP-FGC cells were treated with 30 nM R1881 to generate proliferative shutoff. R1881 (methyltrienolone) is a synthetic, non-metabolized androgen (Roussell-UCLAF, Romainville, France). Exposure to androgen for 24 hours was required to commit LNCaP-FGC cells to an irreversible proliferative shutoff (Geck *et al.*, (1997) *J. Steroid Biochem. Mol. Biol.* 63: 211-218). It was concluded that at this point, the genes responsible for the shutoff were highly induced. LNCaP-FGC cells reversibly arrested by CDHuS were considered as the shutoff-negative control; they were harvested after three days of CDHuS treatment. Total RNA was prepared by the acidic guanidinium-thiocyanate method and polyA$^+$ RNA was purified by using the FastTrack™ kit (Invitrogen, San Diego, CA) (Chomczinsky *et al.*, (1987) *Anal. Biochem.* 162: 156-159).

Double-stranded cDNA pools from R1881-treated cells (R cDNA) and CDHuS-treated cells (CD cDNA) were synthesized using the Copy Kit™ (Invitrogen), with oligo-dT priming. After *AluI* and *RsaI* digestions and adaptor ligations, the constructs were PCR- amplified (GeneAmp Kit™, Perkin Elmer, Foster City, CA). The amplified CD cDNA were digested with *Eco RI*, photobiotinylated (driver cDNA) and hybridized at 20-fold molar excess to an aliquot of non-biotinylated R cDNA. The hybridized non-specific sequences were eliminated by subsequent Streptavidin chromatography. After 3 cycles of selection, the amplified expressed sequence tag (EST) pool of the androgen-induced shutoff AS (R cDNA pool minus CD cDNA pool) sequences was digested with *EcoRI*, cloned into the BlueScript SK™ vector (Stratagene, La Jolla, CA) and transformed into E. coli (OneShot™ strain, Invitrogen).--

The following paragraph beginning at page 74, line 22, has been replaced with the following rewritten paragraph:

--To sequence the identified EST fragments, PCR sequencing reactions were performed using the dsDNA Sequencing System™ (Life Technologies, Gaithersburg, MD). The EST DNA sequences were tested for homology to known DNA sequences using the FASTA and BLAST (National Center for Biotechnology Information, Bethesda, MD) programs. Five inserts were found with no match in GenBank (Geck *et al.*, (1997) *J. Steroid Biochem. Mol. Biol.* 63: 211-218). For further analysis, the mRNA with the highest induction in shutoff positive LNCaP-FGC cells (AS3, >5-6-fold of the 5.3 kb mRNA, and >3-4-fold of the 8 kb isoform) was selected.--

The following two paragraphs beginning at page 75, line 9, have been replaced with the following rewritten paragraphs:

--For the PCR reaction, the Expand High Fidelity™ kit was used and a 1 μl phage suspension as template (Boehringer-Mannheim). A 40 cycle amplification in a Perkin-Elmer 9600 thermocycler resulted in the production of a 1370 bp 5' fragment and a 3250 bp 3' fragment. These PCR products were purified using Qiagen columns, and sequenced by automatic sequencing using a primer walking strategy. The sequencing data showed that the open reading frame in the 5'end fragment did not have an authentic AUG codon.

To search for the missing 5' end of the transcript, the Prostate Specific Marathon Ready cDNA™ preparation from Clontech was used. Amplifications with the Clontech anchored primer and a set of AS3 specific primers resulted in a 419 bp fragment. The DNA was cloned and sequencing data showed that it carried the N-terminal 118 amino acids of the open reading frame. The nucleotide sequence reported herein has been submitted to GenBank under the accession number U95825 (see also, Geck *et al.*, (1999) *J. Steriod Biochem. Mol. Biol.* 68:41-50).

The paragraph beginning at page 76, line 15, has been replaced with the following rewritten paragraph:

--Computer analysis of the AS3 open reading frame was performed using the Translate program of the Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin. β-strand and α-helix structures were calculated by the Chou-Fasman method using PepStructure and PepPlot programs. Motif and profile predictions were calculated using various programs of the Wisconsin Package, or by using remote servers offering sequence analyses of protein functional domains through the Internet. The following remote servers were used: PROWEB available online at the Proweb Project website ~~(http://www.proweb.org)~~; BLOCKS available online at the Blocks WWW Server ~~(http://www.blocks.fhcrc.org)~~; PRODOM available online at the Prodom website ~~(http://www.toulouse.inra.fr/prodom/)~~; PRINTS available online at

the Prints website ~~(http://www.biochem.ucl.ac.uk/cgi-bin/attwood/)~~ and the Protein Kinase Resource available online at the Protein Kinase Resource website ~~(http://www.sdsc.edu/Kinases/)~~.--

The paragraph beginning at page 78, line 6, have been replaced with the following rewritten paragraph:

-- Furthermore, a putative nuclear localization sequence (NLS) (KKFTQVLEDDEKIRK; SEQ ID NO: 6) resembling that of the androgen receptor and DNA polymerase-α was localized at position 547(Zhou *et al.*, (1994) *J. Biol. Chem.* 269:13115-13123; Bouvier *et al.*, (1995 *Mol. Biol. Cell* 6:1697-1705). Further, the C-terminal region of the putative AS3 polypeptide contains several sequence elements that show similarities to DNA binding proteins. Motifs and ProfileScan searches in the Wisconsin Package indicated helix-loop-helix and Homeo-box signature sequences in the area, and a remote search on the BLOCKS server also identified DNA binding block elements in the C-terminal sequences. Still further, it is noted a serine-rich domain at position 1139, and a proline/glycine-rich domain at the 1284 position were also found. The C-terminal domain (about 200 amino acids) is highly charged and arranged in unique repeats of seven alternating acidic and basic domains.--

The paragraph beginning at page 84, line 17, has been replaced with the following rewritten paragraph:

--In order to develop the foregoing novel cell lines in which to demonstrate that AS3 mediates the androgen-induced shutoff effect, an inducible transgene encoding an AS3 antisense transcript (or empty vector as a negative control) was genetically engineered into a retroviral vector backbone for efficient, stable, integration into cells. In particular, the AS3 antisense gene was cloned into the Clontech pRevTRE™ retroviral vector under the control of a tetracycline sensitive promoter. The promoter has seven repeats of the bacterial *tetO* operator sequence upstream of the minimal CMV promoter which can be bound by the tetracycline transactivator (tTA). The tTA is a fusion protein between the bacterial tetracycline repressor and the V16 herpes virus transactivator. The tetracycline transactivator is sensitive for tetracycline such that, in the presence of tetracycline, the transactivator cannot bind the tetracycline promoter so the transgene is "off" and conversely, in the absence of tetracycline, the gene is "on" (*i.e.*, the Tet-Off™ system; see, *e.g.*, Clontech pRevTRE™ manual for further details).--

At the end of the application, the Sequence Listing (pages 1-23) has been replaced with the Substitute Sequence Listing (pages 1-25) provided herewith.

In the Claims:

5. (Amended) An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule comprising ~~a~~ an AS3 (Androgen Shutoff Gene 3) nucleotide sequence which has ~~is~~ at least ~~50% homologous~~ 70% identity to the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

(b) a nucleic acid molecule comprising a fragment of at least 250 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

(c) a nucleic acid molecule which encodes ~~a~~ an AS3 (Androgen Shutoff Gene 3) polypeptide comprising an amino acid sequence having at least about ~~45% homologous~~ 70% identity to the amino acid sequence of SEQ ID NO:2; and

(d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

6. (Amended) An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions of 6x SSC at about 45°C followed by washing.

47. (Amended) A kit for diagnosing a mammal for the presence of a disease involving altered cell proliferation or an increased likelihood of developing a disease involving altered cell proliferation, said kit comprising a material for measuring AS3 (Androgen Shutoff Gene 3) RNA.

48. (Amended) A method of obtaining a AS3 (Androgen Shutoff Gene 3) polypeptide, said method comprising:

(a) providing a cell with DNA encoding a AS3 (Androgen Shutoff Gene 3) polypeptide, said DNA being positioned for expression in said cell;

(b) culturing said cell under conditions for expressing said DNA; and

(c) isolating said AS3 polypeptide whereby an AS3 (Androgen Shutoff Gene 3) polypeptide is obtained.

49. (Amended) A method of isolating a AS3 (Androgen Shutoff Gene 3) gene or portion thereof having sequence identity to human AS3 (Androgen Shutoff Gene 3), said method comprising amplifying by polymerase chain reaction said AS3 (Androgen Shutoff Gene 3) gene or portion thereof using oligonucleotide primers wherein said primers

(a) are each greater than 15 nucleotides in length;

(b) each have regions of complementarity to opposite DNA strands in a region of the nucleotide sequence of SEQ ID NO: 1; and

(c) optionally contain sequences capable of producing restriction endonuclease cut sites in the amplified product; and isolating said AS3 gene or portion thereof whereby an AS3 (Androgen Shutoff Gene 3) or portion thereof is isolated.

51. (Amended) A kit for determining if a subject is at increased risk of developing prostate cancer comprising:

(a) at least one reagent that specifically detects an AS3 (Androgen Shutoff Gene 3) molecule, wherein said reagent is a nucleic acid that can selectively bind to a nucleic acid encoding AS3 (Androgen Shutoff Gene 3) ~~selected from the group consisting of antibodies that selectively bind AS3, and oligonucleotide probes that selectively bind to DNA encoding AS3~~; and

(b) instructions for determining that the subject is at increased risk of developing prostate cancer by

~~(c)~~ detecting the presence or absence of AS3 (Androgen Shutoff Gene 3) in said subject with at least one reagent; and

~~(d)~~ observing whether or not the subject is at increased risk of developing prostate cancer by observing if the presence of AS3 (Androgen Shutoff Gene 3) is or is not detected with said at least one reagent, wherein reduced or absent levels of AS3 (Androgen Shutoff Gene 3) indicates said subject is at increased risk of developing prostate cancer.